

Amendments to the Specification

Please delete the following paragraphs 292, 293, 312-314, 464 and 465.

Please amend the following paragraphs:

At paragraph 7:

[7.] Acute Myelogenous Leukemia (AML) is a heterogeneous group of neoplasms with a progenitor cell that, under normal conditions, gives rise to terminally differentiated cells of the myeloid series (erythrocytes, granulocytes, monocytes, and platelets). As in other forms of neoplasia, AML is associated with acquired genetic alterations that result in replacement of normally differentiated myeloid cells with relatively undifferentiated blasts, exhibiting one or more type of early myeloid differentiation. AML generally evolves in the bone marrow and, to a lesser degree, in the secondary hematopoietic organs. AML primarily affects adults, peaking in incidence between the ages of 15-40 years, but it is also known to affect both children and older adults. Nearly all patients with AML require treatment immediately after diagnosis to achieve clinical remission, in which there is no evidence of abnormal levels of circulating undifferentiated blast cells.

At paragraph 9:

[9.] Several other humanized and chimeric antibodies are under development or are in clinical trials. In addition, a humanized Ig that specifically reacts with CD33 antigen, expressed both on normal myeloid cells as well as on most types of myeloid leukemic cells, was conjugated to the anti-cancer drug calicheamicin, CMA-676 (Sievers *et al.*, *Blood Supplement*, 308, 504a (1997)). This conjugate, known as the drug MYLOTARG[®] Mylotarg[®], has recently received FDA approval (Caron *et al.*, *Cancer Supplement*, 73, 1049-1056 (1994)). In light of its cytolytic activity, an additional anti-CD33 antibody (HumM195), currently in clinical trials, was conjugated to several cytotoxic agents, including the gelonin toxin (McGraw *et al.*, *Cancer Immunol. Immunother*, 39, 367-374 (1994)) and radioisotopes ¹³¹I (Caron *et al.*, *Blood* 83, 1760-1768 (1994)), ⁹⁰Y (Jurcic *et al.*, *Blood Supplement*, 92, 613a (1998)) and ²¹³Bi (Humm *et al.*, *Blood Supplement*, 38:231P (1997)).

At paragraph 20:

[20.] The N-terminal globular domain of GPIb α contains a cluster of negatively charged amino acids. Several lines of evidence indicate that, in transfected CHO cells expressing GPIb-IX complex and in platelet GPIb α , the three tyrosine residues contained in this domain (Tyr-276, Tyr-278, and Tyr-279) undergo sulfation.

At paragraph 29:

[29.] A commercially available monoclonal antibody to human PGSL-1, KPL1, was generated and shown to inhibit the interactions between PGSL-1 and P-selectin and between PGSL-1 and L-selectin. The KPL1 epitope was mapped to the tyrosine sulfation consensus motif of PGSL-1 (YEYLDYD)(SEQ ID NO: 214)(Snapp et al., Blood 91 1:154-164 (1998)). KPL1 recognizes only this particular epitope and does not cross-react with sulfated epitopes present on other cells, such as B-CLL cells, AML cells, metastatic cells, multiple myeloma cells, and the like.

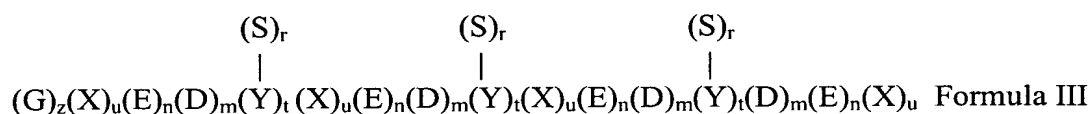
At paragraph 36:

[36.] There are two forms of normal human fibrinogen: fibrinogen γ major and fibrinogen γ prime minor variant, each of which is found in normal individuals. Normal fibrinogen, which is the more abundant form (comprising ~90% of the fibrinogen found in the body), is composed of two identical 55 kDa alpha (α) chains, two identical 95 kDa beta (β) chains, and two identical 49.5 kDa gamma (γ) chains. Normal variant fibrinogen, which is the less abundant form (comprising ~10% of the fibrinogen found in the body), is composed of two identical 55 kDa alpha (α) chains, two identical 95 kDa beta (β) chains, one 49.5 kDa gamma (γ) chain, and one 50.5 kDa gamma prime (γ') chain. The gamma and gamma prime chains are both coded for by the same gene, with alternative splicing occurring at the 3' end. Normal gamma chain is composed of amino acids 1-411. Normal variant gamma prime chain is composed of 427 amino acids: amino acids 1-407 are the same as those in the normal gamma chain, and amino acids 408-427 are VRPEHPAET EYDSL YPEDDL (SEQ ID NO: 220). This region is normally occupied with thrombin molecules.

At paragraph 58:

[58] The present invention provides an isolated epitope comprising the formula

(SEQ ID NO: 216)



Wherein:

- G is Glycine
- E is Glutamate
- D is Aspartate
- Y is Tyrosine
- S is sulfate or a sulfated molecule
- X is any amino acid except the above
- z is 0, 1, or 2
- t is 1, 2 or 3
- r is 0 or 1
- u is 0 to 2
- n is 0 to 3
- m is 0 to 3

wherein at least one Y is sulfated; wherein if $n = 0$ then $m > 0$; wherein if $m = 0$ then $n > 0$; and further wherein the isolated epitope is capable of being bound by an antibody, antigen-binding fragment thereof, or complex thereof comprising an antibody or binding fragment thereof, comprising a first hypervariable region comprising SEQ ID NO: 8 or SEQ ID NO: 20.

At paragraph 68:

[68.] In certain embodiments, antibodies, antibody fragments, and antibody complexes provided according to the present invention are capable of binding to an epitope on a lipid, carbohydrate, peptide, glycolipid, glycoprotein, lipoprotein, and/ or lipopolysaccharide molecule. Preferably, antibodies, antigen-binding fragments thereof, or complexes thereof comprising at least one antibody or binding fragment thereof according to the present invention are capable of binding to lipid, carbohydrate, peptide epitopes, glycolipid epitopes, glycoprotein epitopes, lipoprotein epitopes, and/ or lipopolysaccharide epitopes. In many embodiments, the lipid, carbohydrate, peptide, glycolipid, glycoprotein, lipoprotein, and/ or lipopolysaccharide molecule comprises at least one sulfated moiety.

At paragraph 72:

[72] The present invention provides antibodies, antigen-binding fragments thereof, or complexes thereof comprising at least one antibody or binding fragment thereof are capable of crossreacting with two or more epitopes, each epitope comprising one or more sulfated tyrosine residues within a cluster of acidic amino acids. In certain of these embodiments, antibodies, antigen-binding fragments thereof, or complexes thereof comprising at least one antibody or binding fragment thereof that are capable of crossreacting with at least one cell type selected from the group consisting of B-CLL cells, AML cells, multiple myeloma cells, and metastatic cells. In certain other of these embodiments, antibodies, antigen-binding fragments thereof, or complexes thereof comprising at least one antibody or binding fragment thereof are capable of crossreacting with PSGL-1. Preferably, antibodies, antigen-binding fragments thereof, or complexes thereof comprising at least one antibody or binding fragment thereof that are capable of crossreacting with PSGL-1 bind to the epitope QATEYEYLDYDFLPETE (SEQ ID NO: 225) wherein at least one tyrosine residue is sulfated.

At paragraph 73:

[73] In certain other of these embodiments, antibodies, antigen-binding fragments thereof, or complexes thereof comprising at least one antibody or binding fragment thereof are capable of crossreacting with GP1b- α . Preferably, antibodies, antigen-binding fragments

thereof, or complexes thereof comprising at least one antibody or binding fragment thereof that are capable of crossreacting with GP1b- α bind to the epitope DEGDTDLYDYYPEEDTEGD (SEQ ID NO: 218) wherein at least one tyrosine residue is sulfated, the epitope TDLYDYYPEEDTE (SEQ ID NO: 215) wherein at least one tyrosine residue is sulfated, the epitope GDEGDTDLYDYYP (SEQ ID NO: 270) wherein at least one tyrosine residue is sulfated, the epitope YDYYPEE (SEQ ID NO: 266) wherein at least one tyrosine residue is sulfated, and/or the epitope TDLYDYYP (SEQ ID NO: 267) wherein at least one tyrosine residue is sulfated.

At paragraph 74:

[74.] In yet other of these embodiments, antibodies, antigen-binding fragments thereof, or complexes thereof comprising at least one antibody or binding fragment thereof are capable of crossreacting with fibrinogen γ prime. Preferably, antibodies, antigen-binding fragments thereof, or complexes thereof comprising at least one antibody or binding fragment thereof that are capable of crossreacting with fibrinogen prime $[[\gamma']]$ bind to the epitope E $[[PH]]$ HPAETEDSLYPED (SEQ ID NO: 235) wherein at least one tyrosine residue is sulfated.

At paragraph 76:

[76] In yet other of the se embodiments, antibodies, antigen-binding fragments thereof, or complexes thereof comprising an antibody or binding fragment thereof that are capable of crossreacting with complement compound 4 (CC4) are provided. Preferably, antibodies, antigen-binding fragments thereof, or complexes thereof comprising at least one antibody or binding fragment thereof that are capable of crossreacting with CC4 bind to the epitope MEANEDYEDYEDELPAK (SEQ ID NO: 224) wherein at least one tyrosine residue is sulfated.

At paragraph 78:

[78.] The present invention provides antibodies, antigen-binding fragments thereof, or complexes thereof comprising at least one antibody or binding fragment thereof that are capable of crossreacting with at least one cell type selected from the group consisting of B-CLL cells, AML cells, multiple myeloma cells, and metastatic cells.[:,:]

At paragraph 91:

[91] An isolated GP1b α N-terminal peptide having an apparent molecular weight of about 40 KDa, said peptide comprising an epitope having the sequence YDYYPEE (SEQ ID NO: 266), wherein at least one tyrosine residue in the epitope is sulfated and an isolated GP1b α peptide consisting of amino acids 1 through 282, wherein at least one of amino acids 276, 278 and 279 is sulfated are also provided.

At paragraph 92:

[92.] The present invention also provides polyclonal antibodies, antibody fragments or antibody complexes that cross-react with the variable light chain of human monoclonal antibody scFv Y1. In certain embodiments, the polyclonal antibodies, antibody fragments or antibody complexes cross-react with the peptide encoded by a NdeI-EcoR1 restriction fragment of the variable light chain of human monoclonal antibody Y-1. Diagnostic kits comprising such polyclonal antibodies are also provided.

DEFINITIONS:

At paragraph 93:

[93.] Antibodies (Ab's), or immunoglobulins (Ig[[G]]'s), are protein molecules that bind to antigen. They are composed of units of four polypeptide chains (2 heavy and 2 light) linked together by disulfide bonds. Each of the chains has a constant and variable region. They can be divided into five classes, IgG, IgM, IgA, IgD, and IgE, based on their heavy chain component. The IgG class encompasses several sub-classes including, but not restricted to, IgG₁, IgG₂, IgG₃, and IgG₄. Immunoglobulins are produced *in vivo* by B lymphocytes and recognize a particular foreign antigenic determinant and facilitate clearing

of that antigen.

At paragraph 111:

[111.] Conservative amino acid substitution is defined as a change in the amino acid composition by way of changing one or two amino acids of a peptide, polypeptide or protein, or fragment thereof. The substitution is of amino acids with generally similar properties (e.g., acidic, basic, aromatic, size, positively or negatively charged, polar, non-polar) such that the substitutions do not substantially in a major way alter peptide, polypeptide or protein characteristics (e.g., charge, isoelectric point [[IEF]], affinity, avidity, conformation, solubility) or activity. Typical substitutions that may be performed for such conservative amino acid substitution may be among the groups of amino acids as follows:

- (i) glycine (G), alanine (A), valine (V), leucine (L) and isoleucine (I)
- (ii) aspartic acid (D) and glutamic acid (E)
- (iii) alanine (A), serine (S) and threonine (T)
- (iv) histidine (H), lysine (K) and arginine (R)
- (v) asparagine (N) and glutamine (Q)
- (vi) phenylalanine (F), tyrosine (Y) and tryptophan (W)

At paragraph 113:

[113.] A phagemid is defined as a phage particle that carries plasmid DNA. Phagemids are plasmid vectors designed to contain an origin of replication from a filamentous phage, such as M13 [[m13]] or [[of]] fd. Because it carries plasmid DNA, the phagemid particle does not have sufficient space to contain the full complement of the phage genome. The component that is missing from the phage genome is information essential for packaging the phage particle. In order to propagate the phage, therefore, it is necessary to culture the desired phage particles together with a helper phage strain that complements the missing packaging information.

At paragraph 118:

[118.] An anti-leukemia agent is an agent with anti-leukemia activity. For example, anti-leukemia agents include agents that inhibit or halt the growth of leukemic or immature pre-leukemic cells, agents that kill leukemic or pre-leukemic cells, agents that increase the susceptibility of leukemic or pre-leukemic cells to other anti-leukemia agents, and agents that inhibit metastasis of leukemic cells. In the present invention, an anti-leukemia agent may also be agent with anti-angiogenic activity that prevents, inhibits, retards or halts vascularization of tumors.

At paragraph 124:

[124.] FIG. 3 is an outline of Y1 reactivity with different preparations of platelet GC and membrane fraction 4 of KG-1 cells ~~the optimal determinants for binding of Y1 to its epitope.~~

At paragraph 145:

[145.] FIG. 24 depicts the immunoprecipitation scheme used in the analysis of the specificity of Y1 binding ~~, the results of which are depicted in FIG. Tab 2A, page 17B.~~

At paragraph 153:

[153.] FIG. 33 depicts a Western Blot showing the effect of Aryl-Sulfatase and Mocarhagin cleavage on Y1 binding. ~~FIG. 33 is a graph depicting liver weights (mean +/- SEM) of mice at day 35.~~

At paragraph 159:

[159.] FIG. 39 is a graph illustrating the pharmacokinetics ~~pharmacokinetics~~ of TCA-precipitable radioactivity in plasma after intravenous injection of ¹²⁵I-CONY1 in mice. The sequence of CONY1 is presented at SEQ ID NO: 204.

At paragraph 170:

[170] FIG. 50 provides the amino acid and nucleotide sequences of the Y16 scFv (SEQ ID NOS: 210 & 213).

At paragraph 172:

[172.] FIG. 52 provides the amino acid sequence of the Y1-cys-KAK [[kak]] scFv (SEQ ID NO: 212).

At paragraph 177:

[177.] Proteins extracted from human platelets were Western blot analyzed on SDS-PAGE using the Y1 scFv antibody and the Y17 scFv antibody, in order to identify the epitopes ~~receptors~~ to which the antibodies bind on the surface of the platelets. Using this methodology, it was determined that the Y1 scFv and Y17 scFv epitope on platelets is glyocalicin, one of the subunits of the CD42 complex.

At paragraph 179:

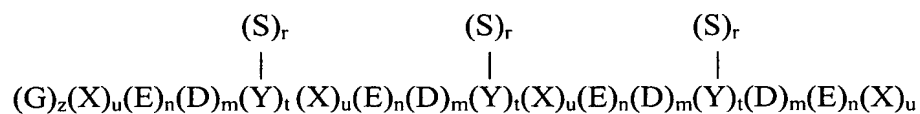
[179.] Based on the results, it was concluded that amino acids 272 through 285 of glyocalicin play a major role in the binding of Y1 to glyocalicin. In addition, since the *E. coli* derived recombinant N-terminal polypeptide of glyocalicin (amino acids 1 to 340 and 1 to 480) was not detectable by the Y1 antibody, it was concluded that Y1 binding to its epitope depends on post-translational modifications, such as glycosylation or sulfation, which are modifications that are not known to occur in *E. coli*[[]].

At paragraph 189:

[189] A preferred epitope is the epitope of Formula II wherein: W is Glycine; Y is a peptide conjugate of Tyrosine or a glyco conjugate of Asparagine, Serine or Threonine; A is Glutamate, γ Carboxy Glutamate or Aspartate, Leucine, Isoleucine Phenylalanine, Serine or Glycine. In certain embodiments, Y is a peptido conjugate of Tyrosine; q is 3; and r is 1.

Formula III:

(SEQ ID NO: 216)



Wherein:

- G is Glycine
- E is Glutamate
- D is Aspartate
- Y is Tyrosine
- S is sulfate or a sulfated molecule
- X is any amino acid except the above
- z is 0, 1, or 2
- t is 1, 2 or 3
- r is 0 or 1
- u is 0 to 2
- n is 0 to 3
- m is 0 to 3

wherein at least one Y is sulfated; wherein if n = 0 then m > 0; wherein if m = 0 then n > 0; and further wherein the isolated epitope is capable of being bound by an antibody, antigen-binding fragment thereof, or complex thereof comprising an antibody or binding fragment thereof, comprising a first hypervariable region comprising SEQ ID NO: 8 or SEQ ID NO: 20.

At paragraph 192:

[192] Such epitopes are found on such diverse molecules as GPIb and PSGL-1 and are found on certain diseased cells, such as B-CLL cells, AML cells, multiple myeloma cells, and metastatic cells. Sulfation of tyrosine and/ or other moieties is particularly important for binding to these epitopes. Human proteins known to be tyrosine sulfated include the following:

Peptide	Sequence
Thrombomodulin (408-426)	ECPEGYILDDGFICTDIDE (SEQ ID NO: 217)
Human GPIb α (269-287)	DEGDTDLYDYYPEEDTEGD (SEQ ID NO: 218)
Human Heparin Cofactor II (56-75)	GEEDDDYLDLEEDDDYIDIVD (SEQ ID NO: 219)
Human Fibrinogen γ' (408-427)	VRPEHPAET EYDSLYPE D [[O]] DL (SEQ ID NO: 220)
α -2-Antiplasmin	PPMEEDYPQFGSP (SEQ ID NO: 221)
Cholecystokinin (CCK)	RISDRDYMGWMD F (SEQ ID NO: 222)
α -2-Choriogonadotropin	CHCSTCY YHKS - COOH (SEQ ID NO: 223)
Complement C4	MEANEDYEDYEYDELP AK (SEQ ID NO: 224)
PSGL-1	QATEY EYLDYDFLPETE (SEQ ID NO: 225)
Factor VIII (716-731)	GDYYEDSYEDISAYLL (SEQ ID NO: 226)
Lumican	GYDYDFPL (SEQ ID NO: 227)

At paragraph 198:

[198.] A second scFv clone, Y17, which binds to platelets and cell lines derived from human myleogenous leukemia cells, particularly AML cells, was also selected. Y17 scFv has the sequence SEQ ID NO: 203. The binding characteristics of Y17 are primarily attributable to its heavy chain CDR3 region, which has the sequence SEQ ID NO: 20. ~~Full Y17-IgG antibodies were also produced.~~

At paragraph 201:

[201.] The hypervariable regions of antibodies of the invention form the antigen binding sites of antibodies of the present invention. The antigen-binding site is complementary to the structure of the epitopes to which the antibodies bind and therefore are referred to as complementarity-determining regions (CDRs). There are three CDRs on each light and heavy chain of an antibody, each located on the loops that connect the β strands of the V_H and V_L domains.[[.]]

At paragraph 219:

[219.] The DNA fragment encoding the V_L domain (variable light chain) of human antibody was PCR-cloned from the Y1 clone (the identical DNA fragment can be obtained from any other clone in the Nissim I library (Nissim et al., "Antibody fragments from a 'single pot' phage display library as immunochemical reagents," *EMBO J.* 13(3): 692-698 (1994)) or even from the human genome using the same methodology) with the following synthetic oligonucleotide primers: oligo 5'-*Nde*I (TTTCATATGGAGCTGACTCAGGACCCTGCT)(SEQ ID NO: 228) and oligo 3'-*Eco*RI (TTTGAATTCCTATTTTGCTTTTGCGGC)(SEQ ID NO: 229). After amplification by polymerase chain reaction (PCR conditions: 94° 1', 56° 2', 72° 2' x30 then 65° 5') the obtained DNA fragment was digested with *Nde*I and *Eco*RI restriction enzymes and cloned into *Nde*I and *Eco*RI restriction enzymes sites of a pre-digested plasmid, which is an IPTG inducible expression vector used for prokaryotic expression of recombinant proteins in *E. coli*. *E. coli* cells were transformed with the ligation mixture and positive clones were

selected by PCR amplification using the above oligonucleotide primers. Cells harboring this plasmid were grown and induced for expression by IPTG. Bacterial cells were harvested by centrifugation from 1 liter of culture post induction with IPTG, inclusion bodies were isolated and solubilized in guanidine-HCl + DTT [[DTE]], and refolded by dilution in a buffer containing Tris-Arginine-EDTA ~~TRIS-ARGININE-EDTA~~. After refolding at 5-10° for 48 hrs, the solution containing protein was dialyzed and concentrated to 20mM Glycine pH 9. The dialyzed solution containing proteins was re-purified by using an ionic exchange column, HiTrapQ, and eluted with a gradient of NaCl. The main peak was analyzed by SDS-PAGE and by gel filtration. At least 10 mgs of purified V_L were obtained from an original 1 liter culture.

At paragraph 220:

[220.] Rabbits were immunized with V_L (400mg) in the presence of CFA (complete Freund's ~~Fruend's~~ adjuvant) then by V_L (200mg) in the presence of IFA (incomplete Freund's ~~Fruend's~~ adjuvant) at 2 to 4 weeks intervals. The titers obtained were low (1:50-1:100) probably due to the high homology between the V_L's from human and rabbit.

At paragraph 222:

[222.] Rabbits were immunized with 400 mg of 1:1 ratio mixture of the purified scFv antibody fragments in the presence of complete Freund's ~~Fruend's~~ adjuvant then with 200 mg of that mixture in the presence of incomplete Freund's ~~Fruend's~~ adjuvant, at 2 to 4 weeks intervals.

At paragraph 228:

[228.] In order to characterize the epitope on the platelet membrane to which Y1 binds, platelet surface proteins were separated by SDS-PAGE (under both reducing and non-reducing conditions) and immunoblotted with biotin labeled-Y1. ~~under reducing and non-reducing conditions.~~ The results of this experiment demonstrate that Y1 reacts with a protein with a molecular mass of 135 kDa under reducing conditions, and with a protein with

molecular mass of ~160 kDa under non-reducing conditions. These molecular masses correspond to platelet GPIb α , which has a molecular mass of 135 kDa under reducing conditions. Under non-reducing conditions, the GPIb α chain disulfide-linked to GPIb β has a molecular mass of 160-kDa. (FIG. 2).

At paragraph 231:

[231.] Western analysis of recombinant GPIb expressed in *E. coli* demonstrated that GPIb expressed in *E. coli* does not react with Y1. Thus, it appears that post-translational modification, which does not occur in *E. coli* is required for Y1 binding. Neither N- nor O-glycanases affect the binding of Y1 to KG-1 cells. However, Y1 binding can be inactivated (eliminated) by treatment of ligands with aryl sulfatases or by proteases. (FIG. 3).

At paragraph 236:

[236.] Washed platelets were treated by molarhagin, and platelet lysates were electrophoresed on SDS-polyacrylamide gels and transferred to nitrocellulose. Western blot analysis of lysates of molarhagin-treated washed platelets with Y1 shows a loss of the band corresponding to GPIb α (135 kDa[[]]) and binding of Y1 to the N-terminal ~45 kDa tryptic fragment. A monoclonal antibody (MCA466S) directed against the C-terminal fragment of GPIb α reacted with the ~95 kDa C-terminal fragment, and a monoclonal antibody (S.C.7071) directed against the N-terminal fragment of GPIb α reacted with the same ~45 kDa fragment that was recognized by Y1. (FIG. 7).

At paragraph 241:

[241.] FACS analysis indicated that Y1 and Y17 have similar binding profiles to platelets and KG-1. In addition, both do not bind to Raji and T2 cells. In contrast, TM1 (SEQ ID NO: 209), Y16 (SEQ ID NO: 210), and Y45 do not bind to any of the above mentioned human cell lines.

At paragraph 246:

[246.] ELISA assays were developed to evaluate the effect of the GPIb derived synthetic peptides on the binding of Y1 to purified glyocalicin. In addition, FACS analysis using washed platelets was carried out. To evaluate the importance of sulfated tyrosine within the Y1 binding site of GPIb, a competitive binding FACS analysis was used. Y1-scFv at a concentration of 1 µg was preincubated with different peptides at concentrations of 2.5 and 200 µM. After a preincubation for 30 minutes at room temperature the mixture was added to a tube containing $\sim 10^7$ washed platelets and the binding of Y1 to the washed platelets was assessed using polyclonal rabbit anti-scFv-PE. The inhibitory effect of the peptides compared to control binding (Y1 alone) was evaluated by measuring the residual binding of Y1 to washed platelets. The peptides and the results are described in Tables 1 and 3, respectively ~~Table 1~~, and are similar to results that were observed using the same peptides in an ELISA assay (Table 2) (~~Table B~~). In both assays, a control level of Y1 binding was determined, as follows. A polystyrene microtiter MaxiSorp ~~maxisorb~~ plate was coated with (a) purified glyocalicin or (b) washed platelets. After extensive washing, 0.5 µg/well of Y1 was added. The plate was then incubated with rabbit anti-scFv followed by addition of anti rabbit -HRP (horse radish peroxidase) and HRP substrate. The level of anti rabbit -HRP binding was measured by the intensity of the color produced, and the level of anti rabbit -HRP binding correlates with the level of binding of anti Y1-scFv and the level of binding of Y1. The optical density was measured at A₄₀₅. Each sample was assayed in duplicate, and the average was calculated.

At paragraph 248:

[248] The five peptides are as follows in Table 1:

Table 1

Peptide Name	Characterization	Sequence
EGR	negative control peptide	REEGRQHFFLLLEGRSSYS (SEQ ID NO: 230)
P-1	residues 268-285 of GPIb α	GDEGDTDLYDYYPEEDTE (SEQ ID NO: 231)
P- 1-S	residues 268-285 of GPIb α	GDEGDTDLY*DY*Y*PEEDTE (SEQ ID NO: 232)

P-2-S	residues 273-285 of GPIb α	TDLY*DY*Y*PEEDTE (SEQ ID NO: 233)
P-3-S	residues 268-280 of GPIb α	GDEGDTDLY*DY*Y*P (SEQ ID NO: 234)

Y* is identical to Y which is sulfated tyrosine.

At paragraph 251:

[251.] These results further support the hypothesis that sulfated tyrosine residues within the specific region are important for Y1 recognition on GPIb. Overall, analysis of N-terminal peptide proteolytic fragments of mocarhagin and cathepsin G suggest that the GPIb α amino acid sequence Tyr276-Glu-282 is or contains an important epitope for binding of Y1. (FIG. 3) (~~FIGS. Tab 1C pages 6 and 7~~). Further characterization indicated that in addition to residues 276-282 (sulfated anionic sequence) of glyocalicin, upstream amino acids 283-285 are involved in the recognition site of Y 1.

At paragraph 255:

[255.] The effect of Y1 (scFv) on aggregation of platelet-rich-plasma (PRP) was tested at various concentrations of Y1. PRP was pre-incubated with Y1 scFv, Y17 scFv, or a control TM-1 scFv ~~sTM-1eFv~~ for 4 min at 37EC before being exposed to ristocetin, an inducer of platelet agglutination and aggregation. A reversible inhibitory effect was observed when scFv was added to PRP prior to the addition of ristocetin, and it was dose dependent.

At paragraph 258:

[258.] Due to its natural structure the full IgG Y1 has two binding sites for ~~for~~ ~~for~~ GPIb α and one binding site for an Fc receptor. It is likely that if full IgG Y1 binds two GPIb α molecules, it will activate platelets and induce platelet agglutination. Furthermore, because platelets have a ~~an~~ ~~an~~ Fc-receptor, Y1-IgG can induce platelet agglutination by binding to GPIb α and to an Fc-receptor, thereby producing platelet agglutination by each IgG

Y1 binding to three platelets. Therefore, the effect of IgG Y1 on aggregation of washed platelets was tested at different concentrations of Y1-IgG in the presence or absence of ristocetin. Induction of platelet aggregation by Y1-IgG was monitored for 4 min at 37°C, followed by addition of ristocetin.

At paragraph 262:

[262.] The results are presented in Table 7 and FIG. 18. No effect on platelet aggregation was seen after the addition of ristocetin: normal platelet aggregation was observed. Y1-IgG at a final concentration of 50 µg/ml induced platelet aggregation in Platelet-Rich-Plasma, before the addition of ristocetin. Y1-IgG at a concentration of 25 µg/ml only partially induced platelet aggregation before the addition of ristocetin. No inhibition ~~induction~~ of platelet aggregation was observed with Y1-IgG concentrations of 10 µg/ml, 4 µg/ml, or 1 µg/ml. Commercial monoclonal antibodies against GPIbα (Pharmigen[[]]), which inhibit platelet aggregation at concentration of 20 µg/ml, did not induce platelet aggregation. Control human IgG- Lambda (Sigma) in the same concentration as Y1-IgG also did not induce platelet aggregation.

Table 7		% inhibition		% aggregation	
		Without Ristocetin	With ristocetin	Without ristocetin	With ristocetin
IgG	Concentration				
Y1-IgG	50 µg/ml	64	0	36	100
Y1-IgG	25 µg/ml	75	8	25	92
Y1-IgG	10 µg/ml	93	10	7	90
Y1-IgG	4 µg/ml	98	5	2	95
Y1-IgG	1 µg/ml	95.5	0.5	0.5	99.5
Human anti-CD42 IgG	20 µg/ml	99.5	0.5	0.5	99.5
Control ristocetin Activation		--	0	--	100

At paragraph 263:

[263.] Antibodies against GPIbα (CD42b) recognize platelet lysate and glycolalicin [and] but not KG-1 cell lysate (a Y1 binding positive myeloid cell line) or RAJI [[Raji]] cell lysate (a B cell line that is negative for Y1 binding at concentrations at which KG-1 cells are

positive for Y1 binding). In contrast, Y1 recognized both glyocalicin, platelet lysate, and KG-1 cells, but not RAJI [[Raji]] cell extract. The negative control scFv-181, did not recognize any of the relevant proteins. (FIG. 20).

At paragraph 265:

[265] Two proteins immunoreacted with Y1 both in normal as well as in leukemia patients plasma. The first is designated H P-ligand 1, which has a molecular mass of ~50 kDa under reducing conditions and >300 kDa under non-reducing conditions and which completely disappears from the serum after coagulation; and (2) H P-ligand 2, which has a molecular mass of ~80 kDa under both reducing and non-reducing conditions and which remains in serum after coagulation. After purification using a Q-Sepharose column reverse phase (RP-HPLC) 2D gel electrophoresis, and peptide mapping, the ~50 kDa ligand was identified as the normal variant of the gamma chain (γ prime) of human fibrinogen. The sequence VRPEHPAETEDSLYPEDDL (SEQ ID NO: 220), is present only in fibrinogen gamma prime, but not the abundant form of fibrinogen gamma, and is similar to GPIb anionic region containing sulfated tyrosine. Most likely this is the binding site for Y1. The ~80 kDa was identified as complement compound 4 (CC4) and Lumican. As above, it contains sulfated tyrosine residues accompanied by a stretch of negatively charged amino acids.

At paragraph 269:

[269.] The table below summarizes the biochemical experiments preformed to characterize and localize the Y1 binding site on KG-1 cells.

Western Blot Analysis with Y1 on SDS-PAGE Reducing Gels

Table 10

Substrate	Treatment	Condition	Reactivity with Y1	Presented in Figure
RP-HPLC KG-	O-Sialo	30' at 37 ⁰ C	Reactivity only	<u>FIG. 22</u>

Substrate	Treatment	Condition	Reactivity with Y1	Presented in Figure
1 membrane fraction	glycoprotein endopeptidase		with the 120kDa form	Tab 2A slide 14
RP-HPLC KG-1 membrane fraction	O-Sialo glycoprotein endopeptidase	4hr at 37 ⁰ C	No reactivity	FIG. 22 Tab 2A slide 14
RP-HPLC KG-1 membrane fraction	aryl-sulfatase	18hr at 22 ⁰ C	No reactivity	FIG. 23 Tab 2A slide 14
RP-HPLC KG-1 membrane fraction	Mocarhagin	7' at 37 ⁰ C	No reactivity	FIG. 33 Tab 2A slide 14
Glycocalicin (GC)	O-Sialo glycoprotein endopeptidase	30' at 37 ⁰ C	Enhanced binding	FIG. 22 Tab 2A slide 14
Heparin – BSA	aryl-sulfatase	18hr at 22 ⁰ C	Binds to Y1 as without treatment	FIG. 23 Tab 2A slide 16

At paragraph 270:

[270.] In summary, following treatment with endopeptidases the Y1 signal is cleaved off and cannot be detected (FIG. 22). Most likely, the fragment containing the Y1 binding site is found on the N-terminus ~~N' terminus~~ and it is too small to be determined under the conditions used in the above experiments. Likewise, following treatment with Mocarhagin, the Y1 signal is cleaved off and cannot be detected (FIG. 33), suggesting that the epitope for Y1 is found on the N' terminus of the ligand. In addition, the results obtained with the aryl-sulfatase which remove sulfate entities from proteins (within the KG-1 cell extract), but not from sugar moieties (on the heparin) further support our hypothesis that sulfate is required for Y1 recognition (FIG. 23). Interestingly, O-Sialo glycoprotein endopeptidase enhanced the Y1 signal in the GC cleavage product. We assume that following this treatment the Y1 binding site, now located at the C-terminus ~~C' terminus~~ is better exposed to the Y1 binding.

At paragraph 276:

[276.] Analysis of binding of scFv Y1 antibodies and anti-CD162 antibodies to diseased cells also illustrates that scFv Y1 has binding characteristics different from those of

anti-CD162 antibodies. Specifically, FACS analysis of Y1 and anti-CD162 binding to Pre-B-ALL, HCL, AML, B-ALL, B-CLL, unclassified leukemia, B-PLL, and multiple myeloma cells from human patients showed the two antibodies have different binding profiles. (Table 11) (Table F). Y1 binds to the leukemic cells in 10 of 12 samples. In contrast, anti-CD162 bound only 5 out of 12 samples. Out of the 12 samples, 5 were found to bind Y1 but not anti-CD162. Thus, it may be concluded that, in leukemic cells, scFv Y1 binds to a ligand other than that recognized by anti-CD162.

Table 11: Leukemia samples -- Analysis of Anti-CD162 versus Y1

Patient #	Disease	<u>Reaction with the Leukemia Cells</u>	
		<u>ScFv Y1</u>	<u>Anti CD162</u>
42291	Pre-B-ALL	+	-
42299	HCL	-	-
42311	AML	+	+
42321	B-ALL	-	-
42323	B-CLL	+	-
42325	Unclassified	+	-
42332	B-CLL	+	-
42352	B-PLL	+	+/-
42330	AML	+	+
42334	MM	+	-
42366	AML	+	+
42370	AML/ALL	+	+/-

At paragraph 277:

[277.] Overall, sulfated-tyrosine containing Y1-binding domains in GPIIb α , fibronectin- γ' Fng- γ prime, and PSGL-1, are DEGDTDLYDYYPEEDTEGD (SEQ ID NO: 218) (amino acids 269-287), EHPAETEYDSLYPED (SEQ ID NO: 235) (amino acids 411-427), and QATEYELDYDFLPETE (SEQ ID NO: 225) (amino acids 1-17), respectively. An additional binding site, with a higher affinity to Y1, is most likely to be expressed on primary

leukemia cells. Interestingly, blood samples that are positive both to scFv Y1 and anti-CD162 were derived from AML patients, while B-cell were negative to anti-CD162.

At paragraph 281:

[281] Thus, it is clear that not every sulfated peptide binds to scFv Y1 to the same extent. Also, significantly, these results demonstrate that only one sulfated tyrosine is necessary for binding, as can be seen with peptides P- Y*YY and P-YY Y*. Further, it can be seen that the amino acid context of the sulfated tyrosines influences Y1 binding. For example, P- Y*YY (containing one sulfated tyrosine in the sequence EY*E) inhibits binding efficiently only at 100μM. In contrast, P-YYY*(containing one sulfated tyrosine in the sequence DY*D) inhibits binding efficiently at 1μM.

Table 12: Sulfated Peptides

<u>Name</u>	<u>Source of Peptide</u>	<u>Sequence</u>	<u>#aa</u>	<u>MW</u>	<u>Sulfation</u>
F-YY	Fibrinogen- γ-prime chain	VRPEHPAETYESLYPEDDL (SEQ ID NO: 236)	20	2389	-
F- Y* Y*	Fibrinogen- γ-prime chain	VRPEHPAET*ESLY*PEDDL (SEQ ID NO: 237)	20	2549	Sulfated
P-YYY	PSGL-1-n- terminus	QATEYEYLDYDFLPETE (SEQ ID NO: 225)	17	2126	-
P- Y*YY	PSGL-1-n- terminus	QATEY*EYLDYDFLPETE (SEQ ID NO: 238)	17	2206	Sulfated
P- Y* Y*Y	PSGL-1-n- terminus	QATEY*EY*LDYDFLPETE (SEQ ID NO: 239)	17	2286	Sulfated
P- Y*Y Y*	PSGL-1-n- terminus	QATEY*EYLDY*DFLPETE (SEQ ID NO: 240)	17	2286	Sulfated
P-Y Y*Y	PSGL-1-n- terminus	QATEYEY*LDYDFLPETE (SEQ ID NO: 241)	17	2286	Sulfated
P-Y Y* Y*	PSGL-1-n-	QATEYEY*LDY*DFLPETE	17	2286	Sulfated

Name	Source of Peptide	Sequence	#aa	MW	Sulfation
	terminus	(SEQ ID NO: 242)			
P-YY Y*	PSGL-1-n-terminus	QATEYEYLDY*DFLPETE (SEQ ID NO: 243)	17	2286	Sulfated
G-YYY	GPIb α	GDEGDTDLYDYYPEEDTE (SEQ ID NO: 231)	18	2126	-
G-Y*Y*Y*	GPIb α	GDEGDTDLY*DY*Y*PEEDTE (SEQ ID NO: 244)	18	2366	Sulfated

Y*=Sulfated Tyrosine

At paragraph 282:

[282.] (1) Y1 resembles L-selectin which recognizes both sulfated protein and sugar moieties, and is distinct from the P-selectin [[]], which recognizes only sulfated proteins. Therefore, it can compete for the binding ~~bonding~~ of both proteins.

At paragraph 284:

[284.] Two human leukemia models were developed in immuno-deficient mice as well as [[in]] assay systems.

At paragraph 287:

[287.] In one experiment, SCID mice were pretreated with 100 mg/kg Cytozen (CTX, Cyclophosphamide for injection, Mead Johnson), and were i.v. injected with 2x10⁷ MOLT-4 cells/mouse, 5 days post treatment with cyclophosphamide. Anti-cancer agents or PBS (negative control animals) were injected i.v. three times/week from day 5 post MOLT-4 cells injection and onward. On day 35, blood was drawn from the animals, the animals were sacrificed, and their livers were excised and weighed. In the untreated, PBS-treated MOLT-4 cell-bearing animals, the liver presented with a very massive tumor growth, and its size was increased 2-3-fold relative to PBS control uninfected animals. In this experiment, there were

five treatment groups:

Table 13

1. Not injected with MOLT-4 cells, PBS treated
2. MOLT-4 injected control, PBS-treated
3. MOLT-4 injected, treated with Y1 scFv (CONY 1), 75 µg/mouse
4. MOLT-4 injected, treated with CONY 1 scFv - Doxorubicin, 75 µg/mouse
5. MOLT-4 injected, treated with Doxorubicin, 0.1 mg/kg.

At paragraph 287:

[287.] ~~In one experiment, SCID mice were pretreated with 100mg/kg Cytoxan (CTX, Cyclophosphamid for injection, Mead Johnson). Eleven days after CTX injection, MOLT-4 cells were injected intravenously into the tail vein. Control mice were injected with PBS alone. One week post MOLT-4 injection mice were injected with CONY1-Doxorubicin, which is a conjugate between scFv CON Y1 polypeptide, having KAK amino acid residues at its carboxy end and doxorubicin via a short organic linker; CONY1, which is a scFv antibody fragment derived from Y1 scFv in which the DNA sequences encoding the myc tag of Y1 were deleted and replaced with a DNA sequence encoding the amino acids lysine, alanine, lysine (KAK); or free Doxorubicin. The mice were injected three times per week for three weeks. Control mice were injected with PBS; and another control group did not receive any treatment. (Table M).~~

Table 13

Number of Mice	Inoculation	Treatment
5	PBS only	--
9	MOLT-4	--
9	MOLT-4	CONY-Dox (2.5 mg/kg)
9	MOLT-4	CONY-Dox (2.5 mg/kg)
8	MOLT-4	Free Dox (0.1 mg/kg)

At paragraph 289:

[289.] The results are depicted in (FIGS. 30, 31 and 32). Massive tumor growths (white nodules) were seen in the livers of all mice injected with MOLT-4 cells. ~~However, livers of mice injected with MOLT-4 and treated with CONY1 or CONY1-Doxorubicin conjugate weighted significantly less than those of mice injected with MOLT-4 and treated with free Doxorubicin or left untreated (FIG. 30).~~

At paragraph 294:

[294.] The liver weights, on day 35, are presented in (FIG. 30) (~~FIG. 33~~). As shown, liver size almost tripled in the tumor-infected mice, negative control PBS treated relative to PBS control, and non-MOLT-4-injected mice. The liver weights of mice treated with a low dose of Doxorubicin were similar to that of PBS treated tumor-infected mice. On the other hand, CONY1 scFv and CONY1 scFv-Doxorubicin conjugate treatments markedly inhibited tumor growth in the liver (much lower liver weights).

At paragraph 295:

[295.] In a second ~~In a third~~ experiment, using the identical SCID/MOLT-4 protocol, there were 6 groups:

1. Not injected with MOLT-4, PBS control ~~PBS control, uninfected MOLT-4 cells~~
2. MOLT-4 injected, treated with PBS ~~PBS-treated Molt control~~
3. MOLT-4 Molt injected group, treated with CONY1 scFv, 75 µg/mouse
4. MOLT-4 Molt injected group, treated with a non-specific scFv antibody derived from the Nissim I library, 75 µg/mouse (control)
5. MOLT-4 Molt injected group, treated with Y1-IgG, 5 µg/mouse. 6. ~~MOLT-4 group, treated with a non-specific human IgG, 5 µg/mouse (control)~~
6. MOLT-4 group, treated with a non-specific human-IgG, 5µg/mouse (control)

At paragraph 299:

[299.] In a first experiment, NOD/SCID mice were pretreated with 100mg/kg CYTOXAN[®]. Four days post CYTOXAN[®] injection, KG-1 cells were injected intravenously into the tail vein of six groups of mice. (Table 14 ~~Table N~~, Groups 2 and 5-9). One group of mice (Table 14 ~~Table N~~, Group 1) was injected with PBS alone (control).

At paragraph 300:

[300.] Beginning 14 [Beginning 19] days post KG-1 injection mice were treated with: CONY1, Doxorubicin, CONY1-Doxorubicin conjugate, or MYLOTARG[®] ~~Mylo[®]targ[®]~~. (MYLOTARG[®] ~~Mylo[®]targ[®]~~ is a monoclonal antibody (anti CD33) conjugated chemically to calcheamicin recently approved by the FDA for treatment of AML patients age 60 and over in a first relapse.) Mice were treated once or three times per week for three weeks. One group (group 2) of KG-1 inoculated mice were left untreated. (Table 14) (~~Table N~~). Two other groups of mice (groups 3 and 4) were injected with KG-1 cells that were previously incubated with CONY1 or 181-scFv (a negative, non-specific control antibody) in serum free RPMI containing 1% BSA at 4EC for 1 h. The antibodies were used at a concentration of 0.25mg scFv/10⁸ cells (75 µg/mouse). Before injection into the mice the preincubated KG-1 cells were washed and resuspended in RPMI. The KG-1 cells in RPMI were inoculated into mice at a concentration of 75 µg scFv/ 0.2 ml RPMI per mouse. Group 3 mice were inoculated with KG-1 + CONY1, and group 4 mice were inoculated with KG-1 + 181-scFv. (Table 14). This treatment (group 3 and 4) was carried out one day after the inoculation of groups 1-2 and 5-9, *i.e.*, at five days after CYTOXAN[®] ~~CYTOXAN~~ injection.

Table 14

# of Mice	Group #	Inoculation	Treatment
9	1	PBS	--
11	2	KG-1	--
9	3	KG-1 + Y1	--
9	4	KG-1 + 181	--
8	5	KG-1	75 µg/mouse (2.5 mg/kg) CONY1, 3 times per week

9	6	KG-1	0.1 mg/kg Doxorubicin, 3 times per week
10	7	KG-1	5 mg/kg Doxorubicin, 1 time per week
11	8	KG-1	75 µg/mouse (2.5 mg/kg) CONY1-Doxorubicin, 3 times per week
9	9	KG-1	0.2 mg/kg <u>MYLOTARG</u> [®] My lotarg [®] , 1 time per week

At paragraph 302:

[302.] The results are depicted in (FIGS. 36 and 37) ~~(FIGS. Tab 6, pages 5 and 6)~~. Nine out of 10 KG-1 cells-injected mice that were treated with 5mg/kg free Doxorubicin (group 7) died within three weeks after treatment initiation.

At paragraph 304:

[304.] Overall, nearly all KG-1 injected mice developed leukemia, with average of 30% KG-1 cells in the bone marrow (as determined by FACS analysis). In general, KG-1 engraftment was confined to the bone marrow. Less than 10% KG-1 cells were found in the blood. In one case a solid tumor was observed on peritoneal wall.

At paragraph 307:

[307.] Mice injected with KG-1 cells pre-incubated *in vitro* with CONY1 or 181-scFv (groups 3 and 4, respectively) had a significantly lower percentage of KG-1 cells in their bone marrow.

At paragraph 308:

[308.] The bone marrow of both mice injected with PBS only (negative control) and mice injected with KG-1 cells and treated with MYLOTARG[®] ~~My~~lotarg[™] (group 9) was free of KG-1 cells. These results demonstrate that this *in vivo* model is a useful model for AML.

At paragraph 309:

[309.] The overall percentage of KG-1 cells found in the blood stream of the various groups was very low overall, with high variation within the groups. It should be noted that one mouse treated with MYLOTARG[®] Mylotarg[™] demonstrated relatively high percentage of KG-1 cells in the blood, but not in bone marrow.

At paragraph 311:

[311.] On the first day of analysis, there was a significant difference between mice injected with KG-1 alone (group 2), which had higher percentage of KG-1 cells in their bone marrow, as compared to mice treated with CONY1-Doxorubicin (group 8). On the third day of analysis this situation was reversed: mice from group 8 had a higher percentage of KG-1 cells in their bone marrow when compared to mice from group 2. This situation may have resulted from the following: A) choosing mice in worse physical condition in the first day, B) proliferation of KG-1 cells in mice from group 8 during the days after treatment termination, and C) the number of mice in each group was too small to generate statistically significant results.

Pharmacokinetics of CONY1 in Mice

At paragraph 328:

[328.] Likewise, it is within the ability of the skilled worker using the guidance provided herein to alter the binding characteristics of an antibody, fragment, or construct to obtain a molecule with more desirable characteristics. For example, once an antibody having a desirable property ~~properties~~ is identified, random or directed mutagenesis may be used to generate variants of the antibody, and those variants may be screened for desirable characteristics.

At paragraph 329:

[329.] Antibodies and fragments according to the present invention may also have a

tag may be inserted or attached thereto to aid in the preparation and identification thereof, and in diagnostics. The tag can later be removed from the molecule. Examples of useful tags include: AU1, AU5, BTag, c-myc, FLAG, Glu-Glu [[Glu]], HA, His6 (SEQ ID NO: 245), HSV, HTTPHH (SEQ ID NO: 246), IRS, KT3, Protein C, S-TAG[®] S-Tag[®], T7, V5, and VSV-G (Jarvik and Telmer, *Ann. Rev. Gen.*, 32, 601-618 (1998)). The tag is preferably c-myc or KAK.

At paragraph 335:

[335] In this system, the phage library (as described herein above) was designed to display scFvs, which can fold into the monovalent form of the Fv region of an antibody. Further, and also discussed herein above, the construct is suitable for bacterial expression. The genetically engineered scFvs comprise heavy chain and light chain variable regions joined by a contiguously encoded 15 amino acid flexible peptide spacer. The preferred spacer is (Gly₄Ser)₃ (SEQ ID NO: 247). The length of this spacer, along with its amino acid constituents provides for a nonbulky spacer, which allows the V_H and the V_L regions to fold into a functional Fv domain that provides effective binding to its target.

At paragraph 339:

[339.] The Y1-cys-KAK [[kak]] was produced in a λ -pL vector in bacteria. Expression in the λ -pL vector was induced by increasing the temperature to 42°C. Inclusion bodies were obtained from induced cultures and semi-purified by aqueous solutions, to remove unwanted soluble proteins. The inclusion bodies were solubilized in guanidine, reduced by DTT [DTE], and refolded *in vitro* in a solution based on arginine/oxidized-glutathione ~~ox-glutathione~~. After refolding, the protein was dialyzed and concentrated by tangential flow filtration to a buffer containing urea [[Urea]]/phosphate buffer. The protein was repurified and concentrated by ionic-chromatography in an SP-column.

At paragraph 345:

[345] Varying the length of the spacers is yet another preferred method of forming dimers, trimers, and triamers (often referred to in the art as diabodies, triabodies and tetrabodies, respectively). Dimers are formed under conditions where the spacer joining the two variable chains of a scFv is shortened to generally 5-12 amino acid residues. This shortened spacer prevents the two variable chains from the same molecule from folding into a functional Fv domain. Instead, the domains are forced to pair with complimentary domains of another molecule to create two binding domains. In a preferred method, a spacer of only 5 amino acids (Gly₄Ser) (SEQ ID NO: 248) was used for diabody construction. This dimer can be formed from two identical scFvs, or from two different populations of scFvs and retain the selective and/or specific enhanced binding activity of the parent scFv(s), and/ or show increased binding strength or affinity.

At paragraph 357:

[357.] More specific examples of linker compounds useful according to the present invention, include:

- a. Dicarboxylic acids such as succinic acid, glutaric acid, and adipic acid;
- b. Maleimido hydrazides such as N-[ε-maleimidocaproic acid] hydrazide ~~N-[ε-maleimidocaproic acid] hydrazide~~, 4-[N-maleimidomethyl]cyclohexan-1-carboxylhydrazide, and N-[κ-maleimidoundecanoic acid] hydrazide ~~N-[κ-maleimidoundecanoic acid] hydrazide~~;
- c. PDPH linkers such as (3-[2-pyridyldithio]propionyl hydrazide) conjugated to sulfurhydryl reactive protein; and
- d. Carboxylic acid hydrazides selected from 2-5 carbon atoms.

At paragraph 358:

[358.] Linking via direct coupling using small peptide linkers is also useful. For example, direct coupling between the free sugar of, for example, the anti-cancer drug doxorubicin and a scFv may be accomplished using small peptides. Examples of small peptides include AU1, AU5, BTag, c-myc, FLAG, Glu-Glu, HA, His6 (SEQ ID NO: 245),

HSV, HTTPHH (SEQ ID NO: 246), IRS, KT3, Protein C, S-TAG[®] ~~S-Tag[®]~~ T7, V5, and VSV-G.

At paragraph 368:

[368.] Other exemplary pharmaceutical agents include doxorubicin (adriamycin), morpholinodoxorubicin, methoxymorpholinyldoxorubicin ~~doxorubicin~~, ~~methoxymorpholinyl~~~~doxorubicin~~ (~~morpholinodoxorubicin~~), ~~adriamycin~~, cis-platinum, taxol, calicheamicin, vincristine, cytarabine (Ara-C), cyclophosphamide, prednisone, daunorubicin, morpholinodaunorubicin, methoxymorpholinyldaunorubicin, idarubicin, fludarabine, chlorambucil, interferon alpha, hydroxyurea, temozolomide, thalidomide and bleomycin, and derivatives and combinations thereof.

At paragraph 377:

[377.] Non-limiting examples of anti-cancer or anti-leukemia pharmaceutical agents include doxorubicin (adriamycin), morpholinodoxorubicin, methoxymorpholinyldoxorubicin ~~doxorubicin~~, ~~adriamycin~~, cis-platinum, taxol, calicheamicin, vincristine, cytarabine (Ara-C), cyclophosphamide, prednisone, daunorubicin, morpholinodaunorubicin, methoxymorpholinyldaunorubicin, idarubicin, fludarabine, chlorambucil, interferon alpha, hydroxyurea, temozolomide, thalidomide and bleomycin, and derivatives thereof, an combinations thereof.

At paragraph 395:

[395] **A leader sequence compatible for a mammalian expression system:** An exchangeable system was designed to allow convenient insertion of elements required for a full IgG molecule. The following complimentary double stranded oligonucleotides encoding a putative leader sequence were synthesized, annealed, and ligated into the *Xho*I site of the pBJ-2 mammalian expression vector (under the SR α 5 promoter).
5'-TCGACCTCATCACCATGGCCTGGGCTCTGCTGCTCCTCACCCCTCCTCACTCAGG
ACACAGGGTCCTGGGCCGAT (SEQ ID NO: 249) and

5'-GATCGATTGCACCAGCTGGATATCGGCCCAGGACCCTGTGTCCTGAGTGAGGA
GGGTGAGGAGCAGCAGAGCCCAGGCCATGGTGATGAGG (SEQ ID NO: 250).

Upstream of the initiation ATG codon, two Kozak elements were included. In addition, an internal *EcoRV* site was introduced between the putative cleavage site of the leader sequence and the *XhoI* site to allow subcloning of the variable regions. This modified vector was designated pBJ-3.

At paragraph 397:

[397] 5'-TTTGATATCCAGCTGGTGGAGTCTGGGGGA (SEQ ID NO: 251) (sense) and 5'-GCTGACCTAGGACGGTCAGCTTGGT (SEQ ID NO: 252) (anti-sense) were used for the V_L PCR reaction. The cDNA product of the expected size of ~350 bp was purified, sequenced and digested with *EcoRV* and *AvrII* restriction enzymes. The same procedure was employed to amplify and purify the V_H cDNA region, using the sense and the anti-sense oligonucleotides 5'-GGGATATCCAGCTG(C/G)(A/T)GGAGTCGGGC (SEQ ID NO: 253) and 5'-GGACTCGAGACGGTGACCAGGGTACCTTG (SEQ ID NO: 254), respectively.

At paragraph 399:

[399] For the constant CL-λ3 region, RT-PCR was performed on mRNA extracted from a pool of normal peripheral B-cells (CD19+ cells) in combination with the sense 5'-CCGTCCTAGGTCAGCCCAAGGCTGC (SEQ ID NO: 255) and the anti-sense 5'-TTTGCGGCCGCTCATGAACATTCTGTAGGGGCCACTGT (SEQ ID NO: 256) oligonucleotides. The PCR product of the expected size (~400 bp) was purified, sequenced, and digested with *AvrII* and *NotI* restriction enzymes.

At paragraph 400:

[400] For the constant IgG1 regions (γ chain), a human B cell clone (CMV - clone #40), immortalized at BTG, was selected for PCR amplification. This clone was shown to secrete IgG1 against human CMV and was also shown to induce ADCC response in *in-vitro* assays. For the CH1-CH3 cDNA, oligonucleotides 5'-ACCGCTCGAGTGC(T/C)TCCACCAAGGGCCCATC(G/C)GTCTTC (SEQ ID NO: 257) (sense) and 5'-TTTGCGGCCGCTCATTTACCC(A/G)GAGACAGGGAGAGGCT (SEQ ID NO: 258) (anti-sense) were synthesized and used for PCR amplification. As described for the CL cDNA encoding sequence, the PCR product of expected size (~1500 bp) was purified, sequenced, and digested with *AvrII* and *NotI* restriction enzymes.

At paragraph 413:

[413.] **Binding of full size IgG-Y1 molecule:** Binding experiments were performed to determine the level of binding of the IgG-Y1 molecule compared to the binding level of the scFv-Y1 molecule. A two-step staining procedure was employed, wherein 5 ng of IgG-Y1 were reacted with both RAJI cells (negative control, FIGs. 44-47a ~~Figure 44~~) and Jurkat cells (Y1 positive cells, FIGs. 44-47b and 44-47c ~~Figure 44~~). For detection, PE-labeled goat anti-human IgG was used (FIGs. 44-47c). Similarly, 1 μ g of scFv-Y1 was reacted with Jurkat cells (FIGs. 44-47b) (~~Figure 44~~), and PE-labeled rabbit anti-scFv was used for detection. Results indicate that both IgG-Y1 and scFv-Y1 bind to Jurkat cells, with approximately 10^3 -fold more scFv-Y1 molecules needed to obtain a level of detection similar to that of the IgG-Y1.

At paragraph 452:

[452] The vector pHEN-Y1, encoding the original Y1, was amplified using PCR for both the V_L and the V_H regions, individually. The sense oligonucleotide 5'-AACTCGAGTGAGCTGACACAGGACCCT (SEQ ID NO: 259), and the anti-sense oligonucleotide 5'-TTTGTCGACTCATTTCTTTTTTGC GGCCGCACC (SEQ ID NO: 260) were used for the V_L PCR reaction. The cDNA product of the expected size of ~350 bp was purified, sequenced, and digested with *XhoI* and *NotI* restriction enzymes.

At paragraph 453:

[453] The same procedure was employed to amplify the V_H region (using the sense oligonucleotide 5'-ATGAAATACCTATTGCCTACGG (SEQ ID NO: 261) and anti-sense oligonucleotide 5'-AACTCGAGACGGTGACCAGGGTACC) (SEQ ID NO: 262). The V_H PCR product was digested with *NcoI* and *XhoI* restriction enzymes. A triple ligation procedure into the pHEN vector, pre-digested with *NcoI-NotI*, was employed. The final vector was designated pTria-Y1.

At paragraph 455:

[455] The pTria-Y1 vector from above was linearized with *XhoI* restriction enzyme, and synthetic complimentary double stranded oligonucleotides (5'-TCGAGAGGTGGAGGCGGT (SEQ ID NO: 263) and 5'-TCGAACCGCCTCCACCTC) (SEQ ID NO: 264) were pre-annealed and ligated into the *XhoI* site, between the Y1-heavy and Y1-light chains. This new vector was designated pDia-Y1. As described for the triabodies, the DNA sequence and protein expression was confirmed.

At paragraph 458:

[458.] The binding of Y1-scFv was compared to that of diabodies and triabodies. In this analysis ~~Figure 44~~, the binding profile of all three forms was very similar, indicating that the above modifications in the molecule did not alter, conceal or destroy the apparent binding affinity of Y1 to its ligand.

At paragraph 461:

[461.] In a second RRA using labeled CONY1, a 100 ng/tube of ¹²⁵I-Y1-IgG was used, and competition was performed with each of the three molecules. The results are provided in Figure 47. This figure shows that the affinity of the S-S dimer was 20 times higher than that of CONY1. A rough estimate of the affinity of CONY 1 in this experiment is 10⁻⁶M. The corresponding affinity of the dimer is, therefore, 5x 10⁻⁸ M.

EXAMPLE 13: Production of Y1-cys-KAK [[kak]] (cysteine dimer)

At paragraph 462:

[462.] One liter of λ pL-y1-cys-KAK [[kak]] bacterial culture was induced at 42°C for 2-3 hrs. This culture was centrifuged at 5000 RPM for 30 minutes. The pellet was resuspended in 180 ml of TE (50mM Tris-HCl pH 7.4, 20mM EDTA). 8 ml of lysozyme (from a 5 mg/ml stock) was added and incubated for 1 hr. 20 ml of 5M NaCl and 25 ml of 25% Triton was added and incubated for another hour. This mixture was centrifuged at 13000 RPM for 60 minutes at 4° C. The supernatant was discarded. The pellet was resuspended in TE with the aid of a tissuemiser (or homogenizer). This process was repeated 3-4 times until the inclusion bodies (pellet) were gray/light brown in color. The inclusion bodies were solubilized in 6M Guanidine-HCl, 0.1M Tris pH 7.4, 2 mM EDTA (1.5 grams of inclusion bodies in 10 ml solubilization buffer provided ~10 mg/ml soluble protein). This was incubated for at least 4 hrs. The protein concentration was measured and brought to a concentration of 10 mg/ml. DTT [[DTE]] was added to a final concentration of 65 mM and incubated overnight at room temperature. Re-folding was initiated by dilution of 10 ml of protein (drop by drop) to a solution containing 0.5 M Arginine, 0.1 M Tris pH 8, 2 mM EDTA, 0.9 mM GSSG. The re-folding solution was incubated at ~10° C for 48 hrs. The re-folding solution containing the protein was dialyzed in a buffer containing 25 mM Phosphate buffer pH 6, 100 mM Urea, and concentrated to 500 ml. The concentrated/dialyzed solution was bound to an SP-sepharose column, and the protein was eluted by a gradient of NaCl (up to 1M).

EXAMPLE 14: ELISA to GC (glycocalicin)

At paragraph 463:

[463.] 100 ml of purified glycocalicin was incubated in a 96 flat well MaxiSorp™ [maxisorp] plates, overnight at 4 degrees celsius. The plate was washed with PBST (PBS+0.05% Tween ~~tween~~) 3 times, then 200 ml of PBST-milk (PBST + 2% Non fat milk),

for 1 hr at room temperature. The plate was washed with PBST, and the monomer or dimer (100 ml) was added in PBST-milk at different concentrations for 1 hr at room temp. Then the plate was washed and anti-V_L polyclonal (derived from immunized rabbits with V_L derived from Y1) (1:100 diluted in PBST-milk) was added for an hour. The plate was washed and anti-rabbit HRP was added for an additional hour. The plate was washed 5 times and 100 :1 TMB substrate was added for approximately 15 minutes then 100 µl of 0.5 H₂SO₄ was added to stop the reaction. The optical density of the plate was measured at 450nm in an ELISA reader.

EXAMPLE 15 [[16]]: Production of tetramers of Y1

At paragraph 466:

[466.] A construct was designed where the following sequence, LNDIFEAQKIEWHE (SEQ ID NO: 268), was added at the C-terminus of the Y1 by PCR and cloning into an IPTG inducible expression vector cassette. The clone was named Y1-biotag. This sequence is a substrate for the enzyme BirA, that in the presence of free biotin, the enzyme is capable of covalently connecting biotin to the lysine (K) residue (Phenotypic analysis of antigen-specific T lymphocytes. Science. 1996 Oct 4;274(5284):94-6, Altman JD et al.). This construct was produced as inclusion bodies in BL21 bacterial cells. Refolding was performed as described previously. Inclusion bodies were solubilized in guanidine-DTT ~~guanidine-DTE~~. Refolding was done by dilution in a buffer containing arginine-Tris [[tris]]-EDTA. Dialysis and concentration was performed followed by HiTrapQ ionic exchange purification.